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Effects of Complex Effluents from the River Raisin on Zooplankton Grazing in Lake Erie

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KEY WORDS: hazard evaluation, functional testing, filtering rate, zooplankton, herbivores, ecosystems, inhibition, stimulation, River Raisin, Lake Erie

Great Lakes planktonic systems, like many other aquatic systems, have been exposed to contaminants for decades. Initial attempts to understand, avoid, and ultimately correct former contamination have resulted in the development of separate functional and structural approaches to measuring sensitive parameters. A forthcoming review will examine the successes and failures of functional versus structural testing with respect to hazards in the Great Lakes Basin [*1*]. Evans and McNaught [*1*] have concluded that tests involving single species exposed to complex effluents allow evaluation of the toxicity of real-world waters. Furthermore, they concluded that the most useful functional tests have been based on characteristics of zooplankton reproduction, quantified as net reproductive rates, R_0, or the population growth rate, r. Few studies have measured the effects of pollutants on herbivore ingestion. It would seem that ingestion is a logical functional indicator of environmental hazards, because decreased grazing may result in reduced fecundity of these herbivores. The filtering rates (in millilitres per animal per hour), when multiplied by the food biomass (in milligrams per millitre), provide a direct measurement of the ingestion rate (in milligrams per animal per hour) and, when used alone, provide an indirect measurement.

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Grazing has been used to quantify the hazards associated with single contaminants and, in rare instances, with complex effluents. Mirza [2] showed that grazing by *Daphnia magna* was reduced by polluted waters. Cooley [3] found that filtering by *Daphnia retrocurva* was reduced when animals were exposed to pulp mill effluents. In central Lake Huron, the grazing rates of herbivores were normal but became depressed an average of 87% in polluted Saginaw Bay [4]. All these examples use grazing to estimate the presence of an environmental pollutant, but none identifies the hazard. When looking at the effects of single isomers of common contaminants, with exposures at realistic environmental levels, tests utilizing measurements of grazing have likewise been successful. A pure isomer, dichlorobiphenyl, had no effect on grazing by adult *Cyclops* and nauplii in Lake Huron [4]; however, the degradation product of this compound reduced grazing by 25%. In a similar fashion, toxaphene reduced grazing in Lake Superior by both *Diaptomus sicilis* (8 to 65%) and *Limnocalanus macrurus* (3 to 57%), depending on exposure levels [5]. Similarly, in laboratory tests a single contaminant (lindane) reduced grazing by herbivores [6]. These studies are easier to interpret than those using mixed effluents, but in ecosystems impacted by man it is necessary to understand the impact of complex effluents, because the Great Lakes, along with other large ecosystems, are impacted in this way.

In this volume on functional hazard testing, the authors of this paper have decided to focus on results of functional tests employing species grazing rates because this test alone was the most effective in identifying environmental hazards in a detailed study of the River Raisin in southern Michigan. Hazard tests employing effects of complex effluents on gross primary productivity, bacterial uptake of acetate, and zooplankton reproduction were less successful, when compared side by side using the same effluents.

Study Area

The study area in the lower River Raisin flows through a heavily industralized complex. It is described in detail elsewhere in this volume. We include a map of the lower river (Fig. 1) to emphasize the relationship of the three important stations where effluents were collected: namely, the Monroe Wastewater Treatment Plant (WWTP), denoted Station 7, Mason Run (Station 8), and the discharge from the Ford Motor Co. (Station 9).

Experimental Procedure for Estimating Inhibition of Grazing

Herbivores (rotifers, cladocerans, and copepods) are the principal converters of phytoplankton into animal protein for utilization by fishes. Contaminants such as polychlorinated biphenyl compounds (PCBs) have been demonstrated to have significantly reduced grazing [7]. The filtering rates by zooplankton on the natural algal community of the Lake Erie ecosystem were used to determine the inhibition of this vital ecosystem function. Inhibition or stimulation was studied using dilutions of complex effluents.

Phytoplankton were collected from Lake Erie and grown in the laboratory in a culture chamber (Percival I-60). These phytoplankton were cultured so that their numbers were about equal to those of the natural environment, although the composition undoubtedly changed. For example, in determinations of filtering by *Diaptomus*, the cell numbers decreased over the time of the experiment from 2.5% (September) to 10.3% (July). The density of specific particles in the environment at the onset of these experiments was 454 to 4776 cells per millilitre [in the range of 3 to 40 μm in equivalent spherical diameter (ESD)].

Dilutions of phytoplankton cultures were made with water from the river/lake series of stations, assuming that it was contaminated. Controls were made using lake waters.

The zooplankton species included those forms dominant in Lake Erie (*Diaptomus* spp., *Cyclops bicuspidatus*, and *Daphnia* spp.). The animals were used only in the months they were abundant. All animals were acclimated for 4 h to phytoplankton foods before the introduction

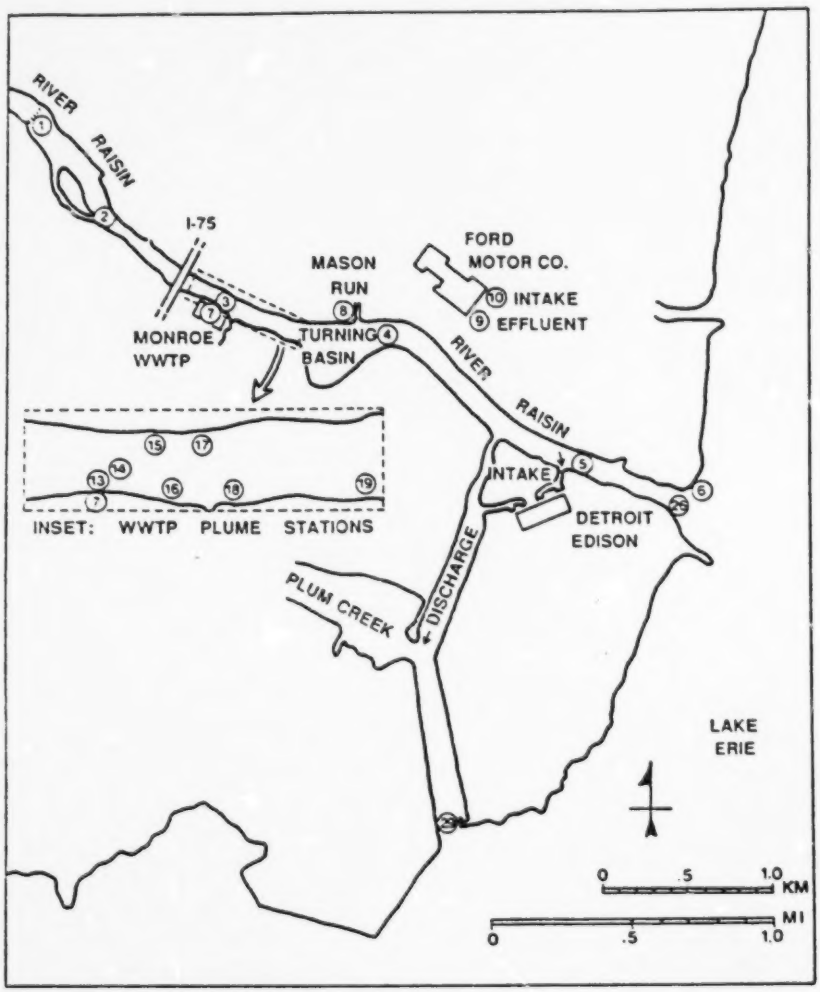

FIG. 1—*The sampling stations in Monroe Harbor on Lake Erie and the River Raisin 1983 and 1984.*

of contaminated or "control" water. Adult animals (ten of a single species) were transferred into experimental feeding vessels (with a food volume of 100 mL) in carriers (tubing with netting to allow the passage of water and food).

Zooplankton grazing, expressed as the filtering rate in these functional estimates, is most accurately measured in the laboratory with a particle counter [*8*]. Calculation of the filtering rates using a presorted size category was done by employing the equation of Gauld [*9*], which requires information on the density of particulate foods, both before and after the introduced animals have fed for 15 min. The filtering rates (in millilitres per animal per hour) represent the volume of water from which particles (within the size category) were removed, produced, or both. The filtering rates were expressed as a function of the equivalent spherical diameter

(ESD) and as a mean for the arbitrary range of 3 to 40 μm ESD. This range of sizes has been used by other investigators as it represents the size of food normally consumed by zooplankton in natural situations.

Results

Functional testing for hazardous substances requires a test or series of measurements that are repeatable with relatively low variability and that represent the impact of contaminants or stimulants on a significant segment of an aquatic system.

Initially, we selected four tests to examine impacts on trophic levels below the level of fishes. All four tests measured single functional responses [*10*] that we commonly understood as major fluxes. These included the bacterial uptake, phytoplankton gross photosynthesis, zooplankton grazing, and zooplankton reproduction (two measurements). The results from the functional tests involving gross photosynthesis are reported elsewhere in this volume (the paper by Bridgham et al.).

The test for inhibition or stimulation of zooplankton grazing proved to be the most valuable of the four tests. A detailed analysis for one month's data is presented showing predominantly inhibition of grazing by complex effluents from three outfalls/streamwaters on the River Raisin (Table 1). These data are for a month (September 1983) when major grazers were present in the natural system, including *Daphnia retrocurva* and *Diaptomus* spp. A summary is presented for 1983 (Figs. 2*a* through 2*c*), showing the consistent relationship between grazing impacted by complex effluents from the WWTP (Station 7), expressed as filtering rates in effluents and control waters from Lake Erie. For one of the months graphed (July), the third major grazer, *Cyclops bicuspidatus*, was present.

Raw data (Table 1) show changes in grazing by these important herbivores, but the responses are varied. Complex effluents from WWTP (Station 7) and Mason Run (Station 8) inhibited grazing by the large calanoid *Diaptomus* and the cladoceran *Daphnia retrocurva*. Grazing by the calanoid was inhibited 65 to 90%, and by the cladoceran 24 to 71%. Neither organism showed increased inhibition with increased dosage. Effluents from the Ford Motor Co. discharge (Station 9) stimulated grazing 14 to 18%. The impact of these effluents on *Diaptomus* is not shown, because in this one case the controls did not feed.

Monthly summaries for an effluent and its impact on grazing show similar changes (Figs. 2*a* through 2*c*). For most dates, except July 1983, sample dilutions from the Monroe WWTP (Station 7) caused an inhibition of zooplankton filtering. For September, filtering by *Diaptomus* and *Daphnia retrocurva* was inhibited in relation to the control filtering; in October, filtering by *Daphnia schodleri* was inhibited; and for April and May 1984, filtering by *Diaptomus* was inhibited. In July 1983, filtering by *Cyclops* and *Diaptomus* was stimulated (Figs. 2*a* and 2*c*).

The reasons for inhibition of secondary ecosystem function by complex effluents from Monroe WWTP are not totally clear. DiToro et al. [*11*] examined the relationship between the zooplankton filtering rate and free residual chlorine and found that the filtering rate increased with the residual chlorine concentration. Furthermore, when filtering was depressed, these events were correlated with high concentrations of zinc and copper [*11*]. Filtering was not compared with concentrations of organic contaminants, which must be considered, since PCBs and toxaphene inhibited grazing [*4,5*].

The filtering rates were likewise inhibited by effluents from Mason Run (Station 8). *Diaptomus* showed inhibited feeding during July and September 1983 and April and May 1984. Most often (July, September, and May) filtering decreased with increased dosage.

For the Ford Motor Co. discharge (Station 9), the filtering rates increased with the dose, showing stimulation. At lower concentrations (10 and 25%), the filtering was equivalent to the control values.

It is possible that inhibition of filtering by herbivores due to contaminants is common in the spring and fall but that stimulation occurs during warmer months. Only one example of this

TABLE 1—*Filtering rates and changes relative to controls for two major herbivores exposed to 10, 25, and 50% additions of complex effluents from three sources on 16 Sept. 1983.*

	Source and Concentration of Complex Effluent								
	WWTP (Station 7)			Mason Run (Station 8)			Ford Motor Co. Discharge (Station 9)		
Herbivore	10%	25%	50%	10%	25%	50%	10%	25%	50%
Diaptomus spp., mL/animal/h (% change relative to controls)	0.14 (−71)	0.16 (−67)	0.17 (−65)	0.03 (−90)	0.03 (−90)	0.03 (−90)	...	...	...
Daphnia retrocurva, mL/animal/h (% change relative to controls)	0.38 (−71)	0.29 (−29)	0.20 (−57)	0.36 (−67)	0.29 (−24)	0.22 (−42)	1.16 (+14)	1.20 (+18)	1.20 (+18)

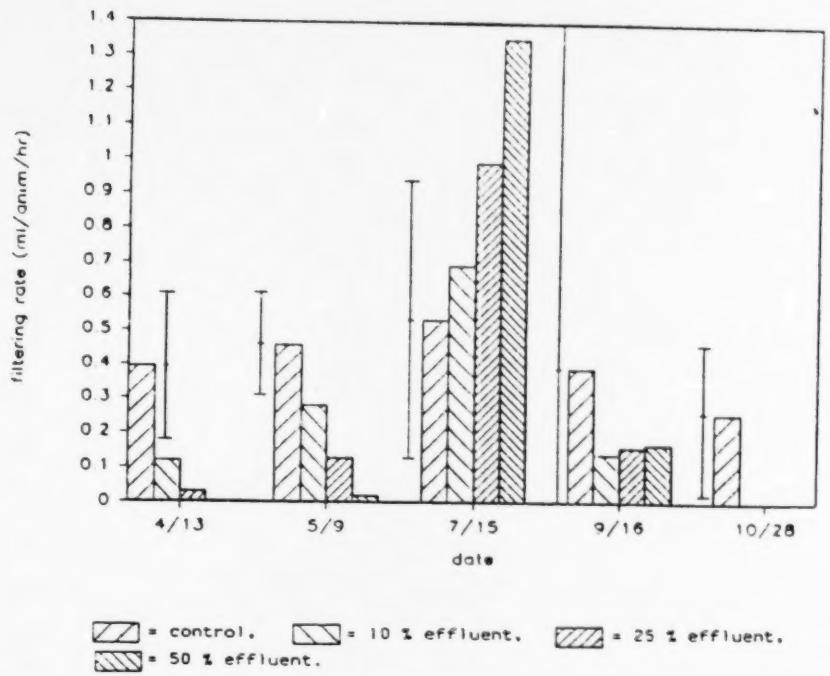

FIG. 2a—*Secondary ecosystem functional bioassay (SEFB) for Monroe WWTP effluents for* Diaptomus. *The range in controls is indicated by the vertical bars.*

phenomenon was available. In July *Diaptomus* was stimulated by all the effluent dilutions and stimulation increased with the dose, whereas in April, May, September, and October, inhibition increased with the dose (Fig. 3).

Discussion

The secondary ecosystem functional bioassay (SEFB) was used in tandem with three other bioassays (involving bacteria, phytoplankton, and zooplankton). It was the only bioassay with limited variability so that hazards presumably related to the occurrence of contaminants could be detected in complex effluents entering a large aquatic ecosystem. Side-by-side comparisons were made with the Mount-Norberg *Ceriodaphnia* bioassay [*12, 13*]. *Ceriodaphnia* life tables were run both for 7 days, as prescribed, and for 40 days or longer to determine the net reproductive rate for each parthenogenic female. The results of these functional tests showed too much variability to detect hazardous substances in River Raisin waters. For the *Ceriodaphnia* test, the standard error (SE) was 19.5% of the mean reproductive rate, R_0. For the gross primary productivity bioassay, the standard error was 44% of the mean rate of primary productivity. For the uptake of acetate by heterotrophs, the standard error was 18% of the mean rate of uptake (Table 2). Thus the SEFB has certain advantages in that it has been demonstrated under field and laboratory conditions to produce consistent answers on specific complex effluents. The SEFB also exhibited some serious problems and was characterized by some costly but not difficult aspects.

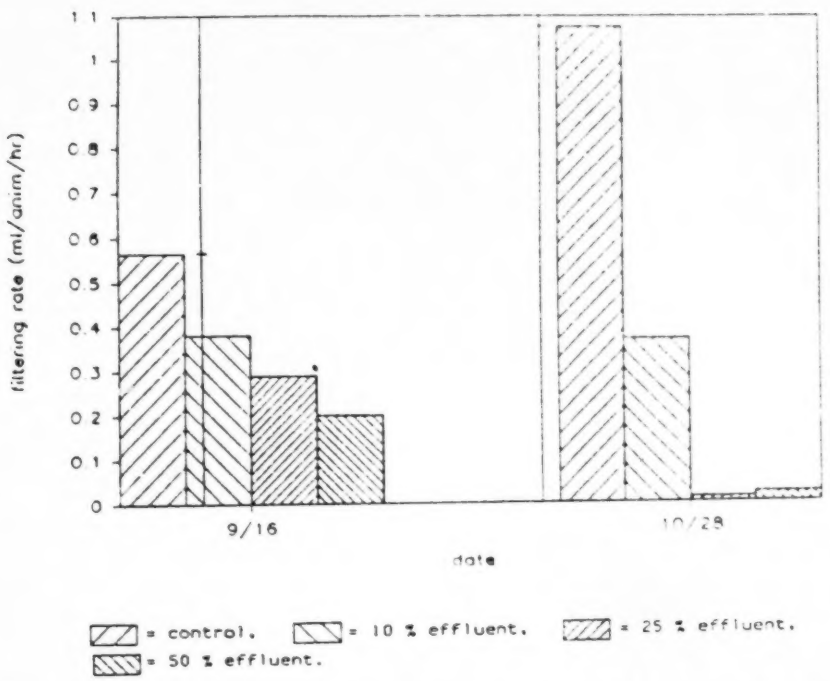

FIG. 2b—*Secondary ecosystem functional bioassay (SEFB) for Monroe WWTP effluents using* Daphnia. *The range in controls is indicated by the vertical bars.*

The most serious problem is the necessity of storing effluents for testing. Effluents should be collected daily, because their toxicity changes in storage,[1] at least with respect to *Ceriodaphnia*. Since locally occurring control waters, with their natural algal assemblages are required, air shipment and maintenance of control water in lighted, temperature-controlled cabinets is a necessity. Control waters and effluents were stored for between 8 days (July) and 33 days (September). This storage may not be as detrimental as thought, because the filtration rate of *Diaptomus* was only depressed slightly. In the May experiment the filtering was depressed 0.5% per day of storage, and in April 0.6% per day. Likewise, natural assemblages of grazers are used. *Diaptomus* and *Cyclops* do not readily reproduce in the laboratory, and adults were collected at the same time as control water samples were taken. *Daphnia* can of course be readily cultured by a variety of methods. All forms must be maintained on native foods or be acclimated to natural assemblages of algae before testing.

Conclusions

1. Zooplankton filtering rates constitute a sensitive and consistent bioassay for complex effluents. The filtering rates respond to an increased dosage of effluents by exhibiting either stimulation or inhibition. This bioassay is called the secondary ecosystem functional bioassay (SEFB).

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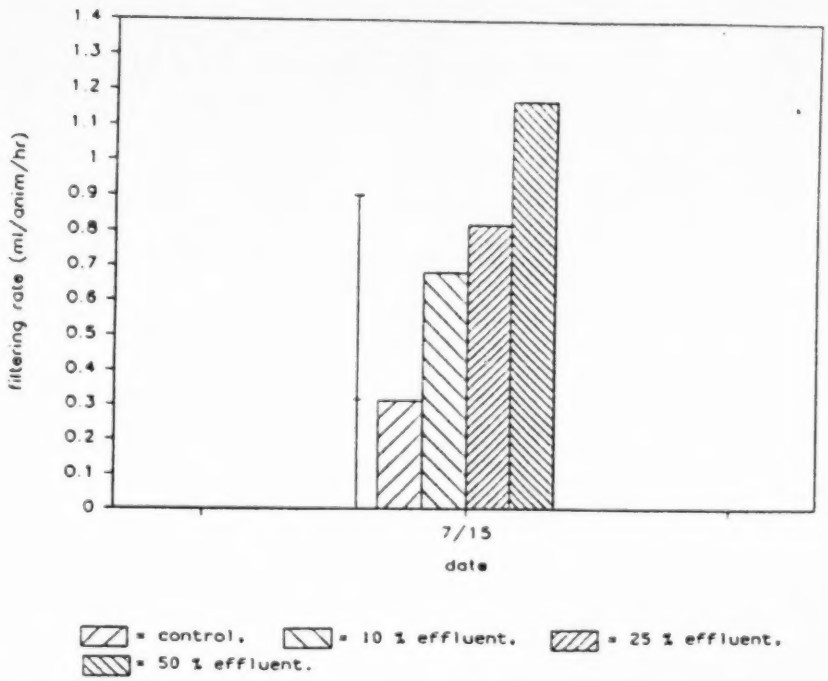

FIG. 2c—*Secondary ecosystem functional bioassay (SEFB) for Monroe WWTP effluents using* Cyclops. *The range in controls is indicated by the vertical bars.*

2. The authors were able to demonstrate the presence of toxic (inhibitory) substances at two locations, including the Monroe WWTP, from a large river system, the River Raisin, using control waters from Lake Erie. The effluents that stimulated the filtration rate came from another industrial source.

3. The inhibition of filtering was due to zinc and copper. In a surprise finding, residual chlorine stimulated the filtering rate.

4. This bioassay utilizing zooplankton filtering rates must be run in the laboratory, is very time-consuming, and involves inherent problems because of the necessity of transporting control water (including algal foods) and animals and the additional problems caused by storing effluents.

5. The SEFB has relatively low variability when directly compared with bioassays involving heterotrophs, phytoplankton, and other zooplankton. We believe that it is an acute test indicative of chronic toxicity, as reproductive effects are logically coupled to ingestive capacity.

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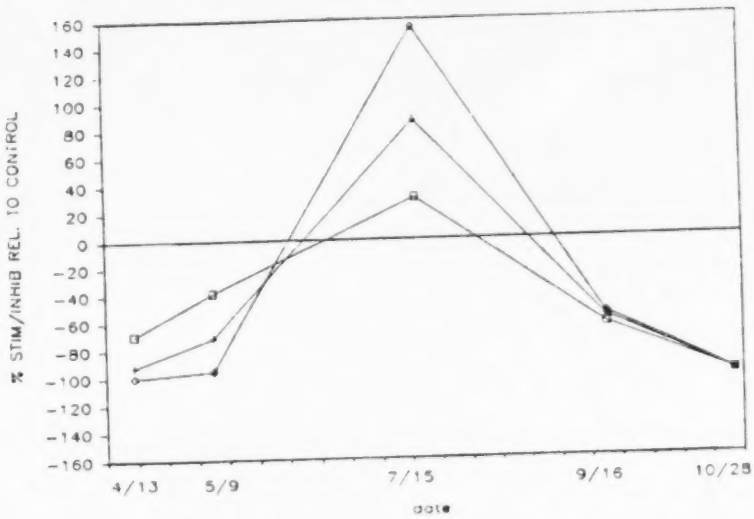

FIG. 3—*Reversal of inhibition with warm, summer weather (July) for the relative filtering rates of* Diaptomus.

TABLE 2—*Comparison of the variability of responses for controls of the four ecosystem indexes.*

Index	Date	Mean Rate (SE)	SE/mean, %	Mean Range	*n*
Filtration rate for *Diaptomus*, mL/animal/h	7/83	0.42 (0.07)	16.7	0.14 to 0.70	8
	9/83	0.51 (0.11)	21.6	0.29 to 1.02	6
	10/83	0.25 (0.03)	12.0	0.16 to 0.38	7
	4/84	0.39 (0.03)	7.7	0.25 to 0.56	12
	5/84	0.45 (0.02)	4.4	0.29 to 0.60	12
Average			12.5		
Net reproductive rate, R_0, for *Ceriodaphnia*, animals/female/life table	all dates	24.0 (4.67)	19.5	13.0 to 54.0	8
Gross primary productivity of Lake Erie community, mg carbon/m^3/h	7/83	90.9 (28.30)	31.1	40.3 to 228.2	6
	9/83	81.3 (2.78)	3.4	73.0 to 85.0	4
	10/83	58.5 (20.54)	35.1	30.6 to 98.6	3
	5/84	33.9 (20.05)	59.1	13.8 to 74.0	3
	6/84	52.2 (27.80)	53.3	24.4 to 80.0	2
	7/84	49.6 (17.49)	35.3	7.8 to 79.0	4
	8/84	41.5 (37.55)	90.5	3.9 to 79.0	2
Average			44.0		
Uptake of acetate by heterotrophs from Lake Erie, μg acetate/L/h	9/83	1.50 (0.26)	17.3	1.14 to 2.00	3
	10/83	−1.17 (2.05)	175.2	−5.26 to 1.05	3
	4/84	0.32 (0.25)	78.1	0.07 to 0.57	2
	5/84	0.65 (0.00)	0	0.65	2
Average			67.7		

son, deserves special thanks. Tim Anderson helped with statistics and graphs, and Terri Alston with manuscript preparation.

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